

AD _____

Award Number: DAMD17-03-1-0466

TITLE: Expression and Promoter Methylation of p16Ink4A During
Estrogen-Induced Mammary Carcinogenesis in the ACI Rat

PRINCIPAL INVESTIGATOR: Lois Beckerbauer Bartsch, Ph.D.

CONTRACTING ORGANIZATION: University of Nebraska Medical Center
Omaha, Nebraska 68198-5100

REPORT DATE: July 2004

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20050113 080

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY
(Leave blank)

2. REPORT DATE
July 2004

3. REPORT TYPE AND DATES COVERED
Annual Summary (1 Jul 03-30 Jun 04)

4. TITLE AND SUBTITLE

Expression and Promoter Methylation of p16Ink4A During Estrogen-Induced Mammary Carcinogenesis in the ACI Rat

5. FUNDING NUMBERS

DAMD17-03-1-0466

6. AUTHOR(S)

Lois Beckerbauer Bartsch, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Nebraska Medical Center
Omaha, Nebraska 68198-5100

E-Mail: lbeckerbauer@unmc.edu

8. PERFORMING ORGANIZATION
REPORT NUMBER

9. SPONSORING / MONITORING
AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING
AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

Original contains color plates. All DTIC reproductions will be in black and white.

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Breast cancer is one of the leading causes of death for women in the United States and estrogen exposure has been hypothesized to be involved in the development of this cancer. Our lab is studying the ACI rat, an estrogen-induced breast cancer animal model to begin to elucidate estrogen's role in breast cancer. The ACI rat develops mammary cancer after prolonged exposure to 17 β -estradiol, while the BN and genetically related COP rats do not. We have identified several polymorphisms in the promoter region of *p16^{cdkn2a}* between the ACI rat and either the BN or COP rats. Several of these polymorphisms are in key regions of transcriptional control for *p16^{cdkn2a}* and similar polymorphisms have been identified in the human. In addition, we have shown in the ACI rat that the *p16^{cdkn2a}* message levels are down regulated and that this down regulation is due to hypermethylation of the promoter region. Further research will focus on the determination of the role of these polymorphisms in the down regulation and methylation of *p16^{cdkn2a}* and the timing of this down regulation and methylation.

14. SUBJECT TERMS

Hypermethylation, P16INK4A, CDKN2A, Tumor Suppressor, Estrogen, Mammary Cancer, Epigenetics

15. NUMBER OF PAGES

18

16. PRICE CODE

17. SECURITY CLASSIFICATION
OF REPORT

Unclassified

18. SECURITY CLASSIFICATION
OF THIS PAGE

Unclassified

19. SECURITY CLASSIFICATION
OF ABSTRACT

Unclassified

20. LIMITATION OF ABSTRACT

Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	7
Reportable Outcomes.....	7
Conclusions.....	8
References.....	8
Appendices.....	9

The ACI rat is uniquely susceptible to estrogen (E2) induced mammary carcinogenesis while the BN and genetically related COP rats are not as susceptible. Genetic studies utilizing these strains have allowed us to identify a region on rat chromosome 5, containing *p16^{cdkn2a}*, that confers susceptibility to E2 induced mammary cancer. *P16^{cdkn2a}* has been identified as a tumor suppressor gene that functions as an inhibitor of CDK4 and CDK6 and controls the G₁/S transition of the cell cycle (1). Loss of *p16^{cdkn2a}* expression has been shown to occur in human breast cancer and is most commonly reported to be due to hypermethylation (1). We have previously demonstrated that the expression of *p16^{cdkn2a}* is dramatically down regulated at the protein level at an early stage of E2-induced mammary carcinogenesis, whether or not this occurs at the mRNA level and the mechanism behind this down regulation has yet to be identified. Our hypothesis is that the mRNA levels of *p16^{cdkn2a}* decrease upon exposure to E2 in the ACI strain in the focal regions of atypical hyperplasia, carcinoma *in situ*, and invasive carcinoma. In addition, we hypothesize that the mRNA levels in the BN and COP strains will not decrease upon the same time of exposure to E2. Furthermore, we hypothesize that this loss of expression is due to methylation of the promoter and exon1 of *p16^{cdkn2a}* in an estrogen dependent manner. To begin to address this hypothesis, we proposed to determine the effect of E2 on the mRNA expression of *p16^{cdkn2a}* in mammary tissue of the ACI, COP and BN strains, to determine the methylation pattern of the promoter and exon 1 of *p16^{cdkn2a}* in the ACI, COP and BN strains of rats in normal, lobular hyperplasia, atypical hyperplasia, carcinoma *in situ*, and invasive carcinoma.

Identification and Characterization of Promoter Polymorphisms in 4 inbred strains of rats. As planned in Task 3 in the statement of work, we sequenced the 5'UTR and promoter region of the ACI/SegHsd, BN/ssNHsd, and COP strains of rats. In addition, we sequenced this same region from the DA/OlaHsd strain of rat. In total we sequenced 1900 bp 5' of the translational start site and have deposited these sequences in GenBank (Accession No AY376712, AY376713, AY376714, and AY376715). Table 1 contains the polymorphisms that have been identified between these three strains of rats (ACI, BN and COP).

Table 1. Comparison of polymorphism identified in four inbred rat strains with mouse and human. Location is respected to translational start site

Location ¹	BN	ACI	COP
-48	ACT	AAT	AAT
-123	15 Cs	11 Cs	11 Cs
-393, --396	CCCCGCCC	CGCCCCC	CCCCGCCC
-400	GCG	GTG	GTG
-578	23 Ts	42 Ts	42 Ts
-588	C - T	CCT	CCT
-589 - 591	CCT	TTT	CCC
-621	CCC	CAG	CAG
-789	TTC	TCC	TTC
-973	ATT	ATT	ACT
-1010	GCG	GTG	GTG
-1423	TCA	TCA	TC-
-1902	TGA	TTA	TTA
-1952	TCT	TTT	TTT
-1971			T insertion
-1998		A deletion	

Rat, mouse (Accession number AF332190) and human (Accession number AF022809) promoter and 5'UTR sequences were compared. The rat 5'UTR and promoter of *p16^{cdkn2a}* has 36% homology with the corresponding human sequence and 80% homology to the mouse sequence. The proximal promoter in human has been identified to contain the first 879 base pairs upstream of the translational start site (2). The homology between rat and human in this area is 41% as compared to the 36% that the entire region exhibits. Several of the polymorphisms identified in the rat are in regions that are homologous to mouse and/or human.

In addition, we compared known binding sites for transcription factors that have been shown to regulated *p16^{cdkn2a}* in the mouse and human. We identified conserved transcription factor binding sites for Ets1 (3), Sp1 (4, 5), RBAR1 (6), RNA helicase A (7), INK4a transcription silence element (8), and E47 (9, 10) in the rat, mouse and human. We also used several transcription factor binding site prediction programs and identified possible binding sites for C/EBP α , HNF-4, PAX4 and ER

transcription factors. Further studies will need to be done to determine if these factors do indeed regulate *p16^{cdkn2a}*.

The transcriptional start site for *p16^{cdkn2a}* in the rat or mouse has yet to be experimentally identified. However, six transcriptional start sites have been identified in the human (2). Comparison of the sequences around these start sites was done. We found homology near three of these sequences in rat and mouse. In addition, Abe et al. (11) identified a putative transcription start site in this same region for the rat.

One important regulatory mechanism that has been identified in the human is hypermethylation of the promoter region. It has been reported that the human *p16^{cdkn2a}* 5'UTR and promoter has a CpG island (12-14). We identified a CpG island located within the rat 5'UTR and promoter using CpGProD (<http://pbil.univ-lyon1.fr/software/cpgprod.html>). We repeated this analysis on human and mouse and in both cases found that they reside in the same portion of the *p16^{cdkn2a}* 5'UTR and promoter region. The identified CpG island includes the translational and transcriptional start sites and conserved transcriptional factor binding sites.

Using the above homologies to mouse and human regions with known and predicted function we chose next to compare the location of the polymorphisms we identified in the rat in relation to these sites. The -48 C/A polymorphism is located near the Sp1 transcription factor binding site. In addition, comparing *p16^{cdkn2a}* promoter and 5'UTR sequences in human we identified a similar polymorphism located in the same position relative to the Sp1 site. In addition, the -393 and -396 G/C polymorphisms lie within the predicted transcriptional start site of the rat and the -393 G/C polymorphism is the residue that has been predicted to be the transcriptional start site in rat. This polymorphism may change the location of the transcriptional start site in the ACI rat relative to the other three strains. This polymorphism also alters the location of a CpG site which also may affect transcriptional initiation. A similar polymorphism exists in human. In addition, the BN sequence has an additional two CpG sites (-400 C/T and -1010 C/T polymorphisms) than does the ACI, COP and DA rat strains, however only the -400 C/T polymorphism lies within the predicted CpG island.

We have begun to prepare a manuscript that reports the comparison of these four sequences to each other. In addition, we plan to include the comparison of the rat sequences to mouse and human and discuss the polymorphisms which fall in similar regions of transcriptional control in this manuscript.

mRNA expression of *p16^{cdkn2a}* is reduced in tumor tissues in comparison to nontumor tissues.

We have started analyzing mRNA expression and methylation of the promoter as outlined in Task 2 and Task 4 in the Statement of work in mammary tissue samples currently available in the Shull Lab. This was done to help develop the experimental approaches necessary to complete these tasks before we begin the animal experiments outlined in Task 1, 2, 4 and 5. More importantly, this will establish if whether *p16^{cdkn2a}* message is down regulated in tumor tissues in comparison to nontumor tissue from the same rats.

To determine whether the observed changes in protein expression was due to a change in *p16^{cdkn2a}* gene expression we used RT-PCR to probe for expression levels of the gene. RNA was isolated from adjacent non-tumor and tumor mammary tissues derived from the same set of E2 treated ACI rats where possible. In total we isolated RNA from 6 non tumor samples from four rats and 24 tumor samples from 18 rats. In some cases multiple non-tumor and /or tumor tissue were analyzed from the same rat. We used primers that amplified from exon 1 of *p16^{cdkn2a}* to exon 3 and were specific for *p16^{cdkn2a}*. Qualitative RT-PCR as shown in Figure 1A and C indicates that expression levels of *S15*, a

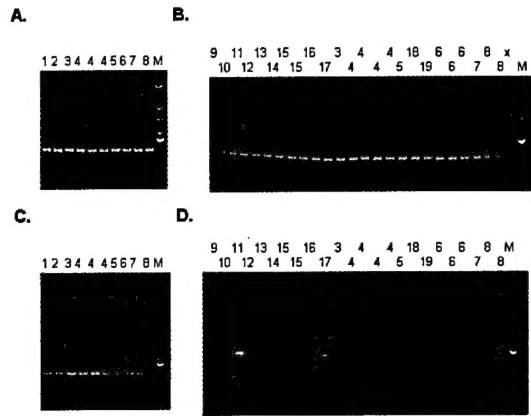


Figure 1. RT-PCR analysis of $p16^{cdkn2a}$ in non-tumor and tumor mammary tissue for 6 rats and tumor tissues from 12 additional rats. *S15* message was used as a positive control (non-tumor, panel A and tumor, panel B). Samples from the non-tumor mammary tissue expressed $p16^{cdkn2a}$ (panel C) while those from tumor samples from the mammary tissue of the same rats did not (Panel D). M is the molecular weight marker. Numbers above lanes refer to the number of rat used. The same number above multiple lanes indicates that multiple tissue samples from the same rat were used. Sample 1 is control RNA from mouse provided by Ambion for use with the Retroscript RTPCR kit. Sample 2 is spleen tissue from an ACI rat.

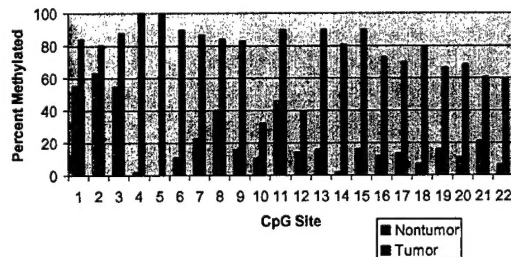


Figure 2. Methylation of the 5'UTR and promoter region in the ACI rat from tumor and non-tumor samples. Methylation is reported in the percent of clones in which methylation was detected (Y axis) at a particular site (X axis).

putative transcriptional start site (sites 4 and 5) was not methylated in the nontumor samples, but was methylated in tumor samples (Figure 2). However, in other areas of the promoter we show little difference between nontumor and tumor samples (Figure 2, sites 1, 2, and 3). The positive control and negative controls for these experiments gave the expected results (data not shown).

We are currently using statistical software to analyze this data to determine which differences in methylation are significant. We are also comparing what is known about $p16^{cdkn2a}$ methylation in humans and in other rat tumors to our results in order to prepare a manuscript on these findings.

housekeeping gene, remained constant in all rats and tissues. By contrast, expression of $p16^{cdkn2a}$, which was present in the non-tumor mammary tissue, was down regulated to below detectable limits in the mammary tumors of the same rats (Figure 1B and D). However, we did identify six tumors that expressed some $p16^{cdkn2a}$. Due to the lack of non-tumor tissue available for these rats, it is not known if the non-tumor of these rats expressed higher levels of $p16^{cdkn2a}$. This data suggests that $p16^{cdkn2a}$ message is down regulated in the tumors for E2 treated ACI rats. Further experiments (Statement of Work Tasks 1 and 2) will help to determine at what histologically discernible lesion this down regulation can first be detected.

Differences in Methylation of DNA from nontumor and tumor tissue from E2 treated ACI rats. DNA was isolated from six tumor and four nontumor samples from five E2 treated ACI rats using Trizol (Gibco-Brl). Four of the rats had DNA isolated from tumor and nontumor tissue and the fifth rat had DNA isolated from two different tumors.

DNA (1.35 ug) was bisulfite treated using the EZ DNA Methylation Kit (Zymo Research). Primers were used to amplify two overlapping portions of the $p16^{cdkn2a}$ promoter region including the putative transcriptional start site and known transcription factor binding sites. As a positive control for methylation, DNA was treated with the enzyme Sss1 Methylase (Promega) that methylates all CpG sites. As a negative control, DNA from spleen tissue from each of the rats was used.

The results from the methylation analysis of the promoter and 5'UTR region of $p16^{cdkn2a}$ was obtained from at least ten clones from three separate bisulfite treatment reactions. Our results indicate that tumor samples were more methylated than the corresponding nontumor sample from the same rat. In addition, the

Key Research Accomplishments

- Sequenced 1900 bp of the 5'UTR and promoter region of *p16^{cdkn2a}* in four strains of rats.
 - Identification of 16 polymorphisms in four strains of rats.
 - Compared rat sequence to mouse and human
 - Compared location of polymorphisms between strains.
- Used RT-PCR to determine expression levels of *P16^{cdkn2a}* in nontumor and tumor tissue
- Used Bisulfite sequencing to determine methylation levels and pattern in nontumor and tumor tissue.

Reportable Outcomes

1. Genebank Submissions (See Appendix)

- Accession No. AY376712. *Rattus norvegicus* strain ACI/SegHsd p16 cyclin dependent kinase inhibitor 2a (*Cdkn2a*) gene, promoter region and partial cds.
- Accession No. AY376713. *Rattus norvegicus* strain BN/ssNHsd p16 cyclin dependent kinase inhibitor 2a (*Cdkn2a*) gene, promoter region and partial cds.
- Accession No. AY376714. *Rattus norvegicus* strain COP p16 cyclin dependent kinase inhibitor 2a (*Cdkn2a*) gene, promoter region and partial cds.
- Accession No. AY376715. *Rattus norvegicus* strain DA/OlaHsd p16 cyclin dependent kinase inhibitor 2a (*Cdkn2a*) gene, promoter region and partial cds.

2. Abstracts

- Bartsch, LM and JD Shull. 2003. Characterization of *p16^{cdkn2a}* As a Genetic Determinate of Mammary Carcinogenesis. Cold Spring Harbor – Rat Genomics and Models Meeting. (See Appendix)
- Beckerbauer, LM, B. Xie, LK Buckles, JD Shull. Potential Early Role of *p16^{cdkn2a}* in Estrogen-Induced Mammary Carcinogenesis in the ACI Rat. 2003 Proceedings of the American Association for Cancer Research. (See Appendix)
- Lachel, CM, LM Bartsch, V. Santos, KL Pennington, CR Murrin, TE Strecker, JD Shull. 2003 Genetic Characterization of Renal Agenesis in the ACI Rat: Mapping of *Renag1* to RNO14. Cold Spring Harbor – Rat Genomics and Models Meeting
- Shull, JD, BS Schaffer, LM Bartsch, LK Buckles, KA Gould, M Tochacek, B Xie, CM Lachel, KL Pennington, TE Strecker, KK Hansen. 2003. Genetic determinants of susceptibility to estrogen-induced mammary cancer in the rat. Breast Cancer Research 5: S10. Presented at the 24th Congress of the International Association for Breast Cancer Research.

3. Manuscripts

- Xie, B., Bartsch LM, Meza JL, McComb RD, Shull JD. 2004. Down-regulation of *Cdkn2a* Expression is an Early Event in Estrogen-Induced Mammary Carcinogenesis in the ACI Rat. Manuscript in Preparation.
- Bartsch LM, Shull JD. 2004. Identification of Polymorphisms in the *p16^{cdkn2a}* 5'UTR and Promoter Sequence in Four Inbred Rat Strains. Manuscript in Preparation.
- Bartsch, LM, Lachel C, Pennington, K, Spady T, Strecker T, Tochacek M, Myer, E, Santos, V, Meza J, Shull, JD. 2004. Identification of a Locus Controlling Unilateral Renal Agenesis in the ACI Rat. Manuscript in Preparation.

Conclusions

We have sequenced the *p16^{cdkn2a}* promoter in four strains of rats and identified 16 different polymorphisms between the strains. Comparing these sequences to known mouse and human sequences we are able to make predictions about the regulation of this gene, which can be further investigated. In addition, a few of the polymorphisms identified in rat have been localized to regions where polymorphisms have been identified in human and therefore may indicate sites in human where differences in regulatory regions may play a role in the etiology of mammary cancer. Several of the polymorphisms add and change the location of CpG sites which may methylate differently and affect the down regulation of *p16^{cdkn2a}*. In addition, our data shows that *p16^{cdkn2a}* is down regulated in mammary tumors from E2 treated ACI rats. This down regulation is similar to that found in human mammary tumors (1). In addition, data from bisulfite sequencing shows that hypermethylation occurs on the *p16^{cdkn2a}* promoter and 5'UTR. This could then lead to the down regulation of *p16^{cdkn2a}*.

Further research (Parts of tasks 1, 2, 4 and 5) will focus on determining the timing of this down regulation, we plan on studying the expression and methylation status of *p16^{cdkn2a}* at early time points of tumor formation, especially in lobular hyperplasia and atypical hyperplasia. In addition to studying the ACI rat we plan on doing the same assays in tissues derived from COP and BN rats. We can then make a correlation between the polymorphisms and methylation and expression status. This will allow us to determine if in the rat these polymorphisms have a functional consequence. If a functional consequence is determined, this may then hold true for E2 induced mammary cancer in humans.

References

1. Rocco, JW, D. Sidransky. 2001. P16 (MTS-1/CDKN2/INK4a) in Cancer Progression. **Exp. Cell Res.** 264: 42-55
2. Hara, E. et al. 1996. Regulation of *p16^{cdkn2a}* Expression and Its Implications for Cell Immortalization and Senescence. **Mol. Cell Biol.** 16: 859-67.
3. Ohtani, N. et al. 2001. Opposing effects of Ets and Id proteins on p16 expression during cellular senescence. **Nature** 409: 1067-70.
4. Pagliuca, A. P. Gallo, L. Lania. 2000. Differential Role for Sp1/Sp3 Transcription Factors in the Regulation of the Promoter Activity of Multiple Cyclin-Dependent Kinase Inhibitor Genes. **J. Cell Biochem.** 76: 360-7.
5. Kurihara, Y. et al. 2002. Induction of p16/INK4a Gene Expression and Cellular Senescence by Toyocamycin. **Biol Phar Bull** 25: 1272-76.
6. Kaneko S., et al. 1999. Transcriptional regulation of CDK inhibitor p16INK4a gene by a novel pRB-associated repressor, RBAR1. **Biochem Mol Biol Int.** 47: 205-15.
7. Myohanen, S., S.B. Baylin. 2001. Sequence-specific DNA Binding Activity of RNA Helicase A to the *p16^{cdkn2a}* Promoter. **J. Biol Chem** 276: 1634-1642.
8. S. Zhang, et al. 2003. *p16^{cdkn2a}* gene promoter variation and differential binding of a repressor, the ras-responsive zinc-finger transcription factor, RREB. **Oncogene** 22: 2285-2295
9. Alani, R.M., A. Z. Young C.B. Shiflett. 2001. Id1 regulation of cellular senescence through transcriptional repression of p16/INK4a. **Proc. Natl. Acad. Sci.** 98: 7812-7816.
10. Zheng, W.J., et al. 2004. Regulation of cellular senescence and *p16^{cdkn2a}* expression by Id1 and E47 proteins in human diploid fibroblast. **J Biol Chem.** Papers in Press. May 11.
11. Abe, M., et al. 2002. Cloning of 5' Upstream Region of the Rat p16 Gene and Its Role in Silencing. **Jpn. J. Cancer Res.** 93: 1100-1106.
12. Soloff, E. V., C.R. Herzog, M. You. 1996. The 5'-flanking region of the E1 α form of the murine *p16^{cdkn2a}* (MTS1) gene. **Gene.** 180: 213-15.
13. Foster, S.A. et al. 1998. Inactivation of *p16^{cdkn2a}* in Human Mammary Epithelial Cells by CpG Island Methylation. **Mol Cell Biol.** 18: 1793-1801.
14. Wong, D.J. et al. 1999. Progressive Region-Specific De Novo Methylation of the *p16* CpG Island in Primary Human Mammary Epithelial Cell Strains during Escape from M₀ Growth Arrest. **Mol Cell Biol.** 19: 5642-5651
15. Holst, C.R., et al. 2003. Methylation of *p16^{cdkn2a}* Promoters Occurs *in vivo* in Histologically Normal Human Mammary Epithelia. **Cancer Res.** 63: 1595-1601.



Entrez PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Nucleotide

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show: 20

Send to

File

Get Subsequence

Fea

☐ 1: AY376712. Rattus norvegicus...[gi:39653664]

Links

LOCUS AY376712 2070 bp DNA linear ROD 01-MAR-2004

DEFINITION Rattus norvegicus strain ACI/SegHsd p16 cyclin dependent kinase inhibitor 2a (Cdkn2a) gene, promoter region and partial cds.

ACCESSION AY376712

VERSION AY376712.1 GI:39653664

KEYWORDS .

SOURCE Rattus norvegicus (Norway rat)

ORGANISM Rattus norvegicus

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Rattus.

REFERENCE 1 (bases 1 to 2070)

AUTHORS Bartsch,L.M. and Shull,J.D.

TITLE Identification of polymorphisms in the p16Cdkn2a 5'UTR and promoter sequence in four inbred rat strains

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 2070)

AUTHORS Bartsch,L.M. and Shull,J.D.

TITLE Direct Submission

JOURNAL Submitted (29-AUG-2003) Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, 985805 Nebraska Medical Center, Omaha, NE 68198, USA

FEATURES Location/Qualifiers

source

1..2070

/organism="Rattus norvegicus"

/mol_type="genomic DNA"

/strain="ACI/SegHsd"

/db_xref="taxon:10116"

/chromosome="5"

/map="5q32"

/tissue_type="spleen"

gene

<1..>2070

/gene="Cdkn2a"

misc_feature

<1..1906

/gene="Cdkn2a"

/note="contains promoter and 5'UTR"

repeat_region

571..590

/rpt_unit="ct"

repeat_region

591..632

/rpt_unit="ca"

repeat_region

1316..1357

/rpt_unit="t"

mRNA

<1907..>2032

/gene="Cdkn2a"

/product="p16 cyclin dependent kinase inhibitor 2a"

CDS

1907..>2032

/gene="Cdkn2a"

/note="p16Cdkn2a"

/codon_start=1

/product="p16 cyclin dependent kinase inhibitor 2a"
/protein_id="AAR29371.1"
/db_xref="GI:39653665"
/translation="MESSADRLARAAALGREHEVRALLEAGASPNAPNTFGRTPIQ"

ORIGIN

```
1  cattggacac attgaccccc ccagagtaat taaatgacta ggtttttctc tgagagttat
61  tttgttagaa cacagggtact ttggggacca cagtgatatg tttagagggt ttaattcccc
121  aaaatagaat tcacaacaag tattagaaga gagtttactt attggtcttt ataacttttg
181  caggaatata cagttatgaa taagggtaaa gaggaattg actttaatgg cctccaccac
241  taaacatgag agaacatgta tgtgttccaa aataataatt tttatttatt gaaaatccac
301  tatatacctg gattttcaca gaattattcaa tactcccaa aatgaccttt cctaggtaaa
361  ttttattttc cttacagacc tcaagagggt tacataattt acttaaacct aaggagagag
421  aacaaagact cagaaaatgt acatagttta tttaaactaa acccagcttg cttggtagca
481  gcctataatc ccagcagttt agaggctgaa gcagggaac ttgggggtata atataagggtg
541  agacttcaca catatacgct ctgtctctgt ctctctctct ctctctctct cacacacaca
601  cacacacaca cacacacaca cacacacaca cagtcagttc tctgtctctc tagttttgtg
661  tccacagcat atgattggca ttctcaccac acacatatgt aaagtcaaaa tagctgtggc
721  catataaaga atatggggag agacaattat tcagcaccta cagaaaataa cgccagggtc
781  ttactcctgg cactcgggag gctcggaaag cagcaggcag agctcctcog agagttagg
841  ttgagttcca ggagagggag aaagggccgc tgccttttcg gtgaggactc ttttttagtc
901  taccggtttg tctagtaatt ttgcccagg cgtgttcttc ctgcctcaca agattgcaat
961  aagtttttaa tgaacaattt aaacctgtgc cattgggtgt gcagtagttc tcaacattta
1021 tgtgcagcat atcaactcat ttaaactcac aacgggtcca taaaatagaa atactttata
1081 agcagattac cctccaaaga ctccactcgc tcgctttttg aaatttgtgt acagaatcct
1141 agcactgcta ccgcaacatc agaaatattt ctgaaaattc ctgcgcaagg ttcggttttc
1201 atacttggtg tagtaccctc caaaataagt tgtttggttg agctagggtt gttgggatct
1261 caccttgggc aggatgtagc tctttcttct gaataaaaaga gaacgcgcct tttccttttt
1321 tttttttttt tttttttttt tttttttttt tttttttgct aagaagttaa atatcttaag
1381 cttcaatttg gcgatgaaaa ctccctcctt cgttttccta aaacctgggg tttgctgggg
1441 aggaggagag atttcgattt cgagcgggta gagaatggac tagttcactt tctcagatgt
1501 gctcctctat ggtgtcgccc ccgaaagga gcccagtacc tcaaggggtg gtcaggcttg
1561 cgggaaaggg tgtcagcgtg ggtagcaggt gggggcagtt cgatccttca gcaagcttc
1621 aactcctagc actccacagg aacctctcac ctctctgca gtccgacccc ggggggtggg
1681 gtgggcagca gtgttttcag aggtgttcaa ttcctgcttt attcagggca catagcgcca
1741 gctaaagcgg gctgtggaac caggtcagga gcaaagcgtg gtttgccccc ccccccgtcc
1801 ccctcagagg aaggaaggag ggccccactg gtcacaccac tggcgactgg gcgggcaactg
1861 aatctccgag aggaaggcga actcgaggag agcgatccgg agcagcatgg agtcctctgc
1921 agatagacta gccagggcag cggccctggg ccgtgagcac gaggtgcggg cactgctgga
1981 agccggggct tcaccaaacg ccccgaaac tttcggtcgt accccgatac aggtaggcaa
2041 ggaataaggg cgaattcgcg gccgctaaat
```

//

[Disclaimer](#) | [Write to the Help Desk](#)
[NCBI](#) | [NLM](#) | [NIH](#)

Jun 8 2004 17:01:12



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Book

Search Nucleotide

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show: 20

Send to

File

Get Subsequence

Fea

☐ 1: AY376713. Rattus norvegicus...[gi:39653666]

Links

LOCUS AY376713 2050 bp DNA linear ROD 01-MAR-2004
 DEFINITION Rattus norvegicus strain BN/ssNHsd p16 cyclin dependent kinase inhibitor 2a (Cdkn2a) gene, promoter region and partial cds.
 ACCESSION AY376713
 VERSION AY376713.1 GI:39653666
 KEYWORDS .
 SOURCE Rattus norvegicus (Norway rat)
 ORGANISM Rattus norvegicus
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Rattus.
 REFERENCE 1 (bases 1 to 2050)
 AUTHORS Bartsch,L.M. and Shull,J.D.
 TITLE Identification of polymorphisms in the p16Cdkn2a 5'UTR and promoter sequence in four inbred rat strains
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 2050)
 AUTHORS Bartsch,L.M. and Shull,J.D.
 TITLE Direct Submission
 JOURNAL Submitted (29-AUG-2003) Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, 985805 Nebraska Medical Center, Omaha, NE 68198, USA
 FEATURES Location/Qualifiers
 source 1..2050
 /organism="Rattus norvegicus"
 /mol_type="genomic DNA"
 /strain="BN/ssNHsd"
 /db_xref="taxon:10116"
 /chromosome="5"
 /map="5q32"
 /tissue_type="spleen"
 gene <1..>2050
 /gene="Cdkn2a"
 misc_feature <1..1886
 /gene="Cdkn2a"
 /note="contains promoter and 5'UTR"
 repeat_region 567..587
 /rpt_unit="ct"
 repeat_region 588..629
 /rpt_unit="ca"
 repeat_region 1312..1334
 /rpt_unit="t"
 mRNA <1887..>2012
 /gene="Cdkn2a"
 /product="p16 cyclin dependent kinase inhibitor 2a"
 CDS 1887..>2012
 /gene="Cdkn2a"
 /note="p16Cdkn2a"
 /codon_start=1

/product="p16 cyclin dependent kinase inhibitor 2a"
/protein_id="AAR29372.1"
/db_xref="GI:39653667"
/translation="MESSADRLARAAALGREHEVRALLEAGASPNAPNTFGRTPIQ"

ORIGIN

```
1 ggacacattg acccacccca gagtaattaa atgactaggt ttttctctga gagttattct
61 gttagaacac aggtactttg gggaccacag tgatatgttt agaggggtga attcccaaaa
121 atagaattca caacaagtat tagaagagag tttacttatt ggtctttata acttttgcag
181 gaatacacag ttatgaataa gggtaaagag gaaattgact ttaatggcct ccaccactaa
241 acatgagaga acatgtatgt gttccaaaat aataattttt atttattgaa aatccactat
301 atacctgat tttcacagaa tattcaatac tccccaaaat gacctttcct aggtaaattt
361 ttttttctct acagacctca agagggtttac ataatttact taaacctaa gagagagaac
421 aaagactcag aaaatgtaca tagtttattt aaactaaacc cagcttgctt ggtagcagcc
481 tataatccca gcagtttaga ggctgaagca gggcaacttg gggataata taaggtgaga
541 cttcacacat atacgctctg tctctgtctc tctctctctc tctctctcac acacacacac
601 acacacacac acacacacac acacacacag tcagttctct gtctctctag ttttgtgtcc
661 acagcatatg attggcattc tcaccacaca catatgtaa gtcaaaatag ctgtggccat
721 ataaagaata tggggagaga caattattca gcacctacag aaaataacgc caggtcttta
781 ctctctggcag tcgggaggct cggaaagcag caggcagagc tcctccgaga gtttaggttg
841 agttccagga gagggagaaa gggccgctgc cttttcggcg aggactcttt tttagtctac
901 ccgtttgtct agtaattttg cccaaagcgt gttcttctct cctcacaaga ttgcaataag
961 ttttaaataga acaatttaaa cctgtgccat tgggtgtgca gtagttctca acatttatgt
1021 gcagcatatc aactcattta aactcacaac gggccataa aatagaaata ctttataagc
1081 agattaccct ccaaagactt cactcgctcg ctttttgaaa tttgtgtaca gaatcctagc
1141 actgctaccg caacatcaga aatatttctg aaaattcctc gccagggttc gggtttcata
1201 cttggtatag taccctccaa aataagttgt ttgtttgagc tagggttgtt gggatctcac
1261 cttggcccg atgtagctct ttcttctgaa taaaagagaa cgcgccttcc cttttttttt
1321 tttttttttt ttttgctaag aagttaaata tottaagctt caatttggcg atgaaaactc
1381 cctccttcgt tttcctaaaa cctgggggtg ctggggagga ggagagattt cgatttcgag
1441 cgggtagaga atggactagt tcactttctc agatgtgctc ctctatggcg tccccgcccg
1501 aaaggagccc agtacctcaa ggggtggtca ggcttgcggg aaagggtgtc agcgtgggta
1561 gcagggtggg gcagttcgat ccttcagcga agcttcaact cctagcactc cacaggaacc
1621 cctcacctcc tctgcagtcc gaccccgggg ggtgggggtg gcagcagtgt tttcagaggt
1681 gttcaattcc tgctttattc agggcacata gcgccagcta aagcgggctg tggaaaccagg
1741 tcaggagcaa agcgtggttt gccccccccc ccccccgctc ccctcagagg aaggaaggag
1801 ggccccactg gtcacaccac tggcgactgg gcgggactg actctccgag aggaaggcga
1861 actcgaggag agcgatccgg agcagcatgg agtcctctgc agatagacta gccagggcag
1921 cggccctggg ccgtgagcac gaggtgcggg cactgctgga agccggggct tcaccaaacg
1981 cccggaacac tttcggtcgt accccgatac aggtaggcaa ggaataaggg cgaattcgcg
2041 gccgctaaat
```

//

[Disclaimer](#) | [Write to the Help Desk](#)
[NCBI](#) | [NLM](#) | [NIH](#)

Jun 8 2004 17:01:12



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Books

Search Nucleotide

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show: 20

Send to

File

Get Subsequence

Fea

☐ 1: AY376714. *Rattus norvegicus*...[gi:39653668]

Links

LOCUS AY376714 2070 bp DNA linear ROD 01-MAR-2004

DEFINITION *Rattus norvegicus* strain COP p16 cyclin dependent kinase inhibitor 2a (Cdkn2a) gene, promoter region and partial cds.

ACCESSION AY376714

VERSION AY376714.1 GI:39653668

KEYWORDS .

SOURCE *Rattus norvegicus* (Norway rat)ORGANISM *Rattus norvegicus*Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; *Rattus*.

REFERENCE 1 (bases 1 to 2070)

AUTHORS Bartsch,L.M. and Shull,J.D.

TITLE Identification of polymorphisms in the p16Cdkn2a 5'UTR and promoter sequence in four inbred rat strains

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 2070)

AUTHORS Bartsch,L.M. and Shull,J.D.

TITLE Direct Submission

JOURNAL Submitted (29-AUG-2003) Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, 985805 Nebraska Medical Center, Omaha, NE 68198, USA

FEATURES Location/Qualifiers

source

1..2070

/organism="Rattus norvegicus"

/mol_type="genomic DNA"

/strain="COP"

/db_xref="taxon:10116"

/chromosome="5"

/map="5q32"

/tissue_type="spleen"

gene

<1..>2070

/gene="Cdkn2a"

misc_feature

<1..1905

/gene="Cdkn2a"

/note="contains promoter and 5'UTR"

repeat_region

571..632

/rpt_unit="ct"

repeat_region

591..632

/rpt_unit="ca"

repeat_region

1316..1357

/rpt_unit="t"

mRNA

<1906..>2031

/gene="Cdkn2a"

/product="p16 cyclin dependent kinase inhibitor 2a"

CDS

1906..>2031

/gene="Cdkn2a"

/note="p16Cdkn2a"

/codon_start=1

/product="p16 cyclin dependent kinase inhibitor 2a"
/protein_id="AAR29373.1"
/db_xref="GI:39653669"
/translation="MESSADRLARAAALGREHEVRALLEAGASPNAPNTFGRTPIQ"

ORIGIN

```
1 ttggacacat tgaccacccc cagagtaatt aaatgactat ggttttttctc tgagagttat
61 tttgttagaa cacaggtact ttggggacca cagtgatatg tttagagggt ttaattcccc
121 aaaatagaat tcacaacaag tattagaaga gagtttactt attggtcttt ataacttttg
181 caggaatata cagttatgaa taagggtaaa gaggaattg actttaatgg cctccaccac
241 taaacatgag agaacatgta tgtgttccaa aataataatt tttatttatt gaaaatccac
301 tatatacctg gattttcaca gaattattcaa tactccccaa aatgaccttt cctaggtaaa
361 ttttattttc cttacagacc tcaagagggt tacataattt acttaaacct aaggagagag
421 aacaaagact cagaaaatgt acatagttta tttaaactaa acccagcttg cttggttagca
481 gcctataatc ccagcagttt agaggctgaa gcagggaac ttgggggtata atataagggtg
541 agacttcaca catatacgct ctgtctctgt ctctctctct ctctctctct ccacacacac
601 acacacacac acacacacac acacacacac agtcagttct ctgtctctct agttttgtgt
661 ccacagcata tgattggcat tctcaccaca cacatatgta aagtcaaaat agctgtggcc
721 atataaagaa tatggggaga gacaattatt cagcacctac agaaaataac gccagggtctt
781 tactcctggc actcggggagg ctcggaagc agcaggcaga gtcctccga gagtttaggt
841 tgagttccag gagagggaga aaggggcgtt gccttttcgg tgaggactct tttttagtct
901 acccgtttgt ctagtaactt tgcccaaagc gtgttcttcc tgctcaca gattgcaata
961 agtttttaaa gaacaattta aacctgtgcc attgggtgtg cagtagttct caacatttat
1021 gtgcagcata tcaactcatt taaactcaca acgggtccat aaaatagaaa tactttataa
1081 gcagattacc ctccaaagac ttcactcgct cgctttttga aatttggtga cagaatccta
1141 gcaactgtac cgcaacatca gaaatatttc tgaaaattcc tcgccaaggt tcgggtttca
1201 tacttggtat agtaccctcc aaaataagtt gtttgtttga gctagggttg ttgggatctc
1261 accttgcca ggatgtagct ctttcttctg aataaaaagag aacgcgcctc cccctttttt
1321 tttttttttt tttttttttt tttttttttt ttttttgcta agaagttaa tatcttaagc
1381 ttcaatttgg cgatgaaaac tccctccttc gttttcctaa aacctggggt ttgctgggga
1441 ggaggagaga tttcgatttc gagcgggtag agaattggact agttcacttt ctcagatgtg
1501 ctctctatg gtgtccccgc ccgaaaggag ccagtagctt caaggggtgg tcaggcttgc
1561 gggaaagggt gtcagcgtgg gtagcaggtg ggggcagttc gatccttcag cgaagcttca
1621 actcctagca ctccacagga acccctcacc tcctctgcag tccgaccccg ggggggtggg
1681 tgggcagcag tgttttcaga ggtgttcaat tcctgcttta ttcagggcac atagcgccag
1741 ctaaagcggg ctgtggaacc aggtcaggag caaagcgtgg tttgcccccc ccccggtccc
1801 cctcagagga aggaaggagg gccccactgg tcacaccact ggcgactggg cgggcaactga
1861 atctccgaga ggaaggcgaa ctcgaggaga gcgatccgga gcagcatgga gtcctctgca
1921 gatagactag ccagggcagc ggcctggggc cgtgagcacg aggtgcgggc actgctggaa
1981 gccggggctt caccaaagc cccgaacact ttcggtcgta ccccgatata ggtaggcaag
2041 gaataagggc gaattcgcgg ccgctaaatt
```

//

[Disclaimer](#) | [Write to the Help Desk](#)
[NCBI](#) | [NLM](#) | [NIH](#)

Jun 8 2004 17:01:12



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Books

Search Nucleotide

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show: 20

Send to

File

Get Subsequence

Fea

☐ 1: AY376715. Rattus norvegicus...[gi:39653670]

Links

LOCUS AY376715 2060 bp DNA linear ROD 01-MAR-2004

DEFINITION Rattus norvegicus strain DA/OlaHsd p16 cyclin dependent kinase inhibitor 2a (Cdkn2a) gene, promoter region and partial cds.

ACCESSION AY376715

VERSION AY376715.1 GI:39653670

KEYWORDS .

SOURCE Rattus norvegicus (Norway rat)

ORGANISM Rattus norvegicus

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Rattus.

REFERENCE 1 (bases 1 to 2060)

AUTHORS Bartsch,L.M. and Shull,J.D.

TITLE Identification of polymorphisms in the p16Cdkn2a 5'UTR and promoter sequence in four inbred rat strains

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 2060)

AUTHORS Bartsch,L.M. and Shull,J.D.

TITLE Direct Submission

JOURNAL Submitted (29-AUG-2003) Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, 985805 Nebraska Medical Center, Omaha, NE 68198, USA

FEATURES Location/Qualifiers

source

1..2060

/organism="Rattus norvegicus"

/mol_type="genomic DNA"

/strain="DA/OlaHsd"

/db_xref="taxon:10116"

/chromosome="5"

/map="5q32"

/tissue_type="spleen"

gene

<1..>2060

/gene="Cdkn2a"

misc_feature

<1..1906

/gene="Cdkn2a"

/note="contains promoter and 5'UTR"

repeat_region

571..590

/rpt_unit="ct"

repeat_region

591..632

/rpt_unit="ca"

repeat_region

1316..1357

/rpt_unit="t"

mRNA

<1907..>2032

/gene="Cdkn2a"

/product="p16 cyclin dependent kinase inhibitor 2a"

CDS

1907..>2032

/gene="Cdkn2a"

/note="p16Cdkn2a"

/codon_start=1

/product="p16 cyclin dependent kinase inhibitor 2a"
/protein_id="AAR29374.1"
/db_xref="GI:39653671"
/translation="MESSADRLARAAALGREHEVRALLEAGASPNAPNTFGRTPIQ"

ORIGIN

```
1 cattggacac attgaccccc ccagagtaat taaatgacta ggtttttctc tgagagttat
61 tttgttagaa cacaggtact ttggggacca cagtgatatg tttagagggt ttaattcccc
121 aaaatagaat tcacaacaag tattagaaga gagtttactt attggtcttt ataacttttg
181 caggaatata cagttatgaa taagggtaaa gaggaattg actttaatgg cctccaccac
241 taaacatgag agaacatgta tgtgttccaa aataataatt tttatttatt gaaaatccac
301 tatatacctg gattttcaca gaatattcaa tactcccaa aatgaccttt cctaggtaaa
361 ttttattttc cttacagacc tcaagagggt tacataattt acttaaacct aaggagagag
421 aacaaagact cagaaaatgt acatagttta tttaaactaa acccagcttg cttggtagca
481 gcctataatc ccagcagttt agaggctgaa gcagggaac ttgggggtata atataagggtg
541 agacttcaca catatacgtc ctgtctctgt ctctctctct ctctctctct cacacacaca
601 cacacacaca cacacacaca cacacacaca cagtcagttc tctgtctctc tagttttgtg
661 tccacagcat atgattggca ttctcaccac acacatatgt aaagtcaaaa tagctgtggc
721 catataaaga atatggggag agacaattat tcagcaccta cagaaaataa cgccaggctc
781 ttactcctgg cactcgggag gctcggaaag cagcaggcag agctcctccg agagtttagg
841 ttgagttcca ggagagggag aaagggccgc tgccttttcg gtgaggactc ttttttagtc
901 taccggtttg tctagtaatt ttgccaaaag cgtgttcttc ctgcctcaca agattgcaat
961 aagtttttaa tgaacaattt aaacctgtgc cattgggtgt gcagtagttc tcaacattta
1021 tgtgcagcat atcaactcat ttaaaactcac aacgggtcca taaaatagaa atactttata
1081 agcagattac cctccaaaga ctccactcgc tcgctttttg aaatttgtgt acagaatcct
1141 agcactgcta ccgcaacatc agaaatattt ctgaaaattc ctcgccaagg ttcggttttc
1201 atacttggtg tagtaccctc caaaataagt tgtttgtttg agctagggtt gttgggatct
1261 caccttggcc aggatgtagc tctttcttct gaataaaaaga gaacgcgcct tttccttttt
1321 tttttttttt tttttttttt tttttttttt tttttttgct aagaagttaa atatcttaag
1381 cttcaatttg gcgatgaaaa ctccctcctt cgttttccta aaacctgggg tttgctgggg
1441 aggaggagag atttcgattt cgagcgggta gagaatggac tagttcactt tctcagatgt
1501 gctcctctat ggtgtccccg cccgaaagga gcccagtagc tcaaggggtg gtcaggcttg
1561 cgggaaaggg tgtcagcgtg ggtagcaggt gggggcagtt cgatccttca gcgaagcttc
1621 aactcctagc actccacagg aacctctcac ctctctgca gtccgacccc ggggggtggg
1681 gtgggcagca gtgttttcag aggtgttcaa ttctgctttt attcagggca catagcgcca
1741 gctaaagcgg gctgtggaac caggtcagga gcaaagcgtg gtttgccccc ccccccgtcc
1801 ccctcagagg aaggaaggag ggccccactg gtcacaccac tggcgactgg gggggcactg
1861 aatctccgag aggaaggcga actcgaggag agcgatccgg agcagcatgg agtcctctgc
1921 agatagacta gccagggcag cggccctggg ccgtgagcac gaggtgcggg cactgctgga
1981 agccggggct tcaccaaacg ccccgaaacac ttctggtcgt accccgatac aggtaggcaa
2041 ggaataaggg cgaattcgcg
```

//

[Disclaimer](#) | [Write to the Help Desk](#)
[NCBI](#) | [NLM](#) | [NIH](#)

Jun 8 2004 17:01:12

IDENTIFICATION AND CHARACTERIZATION OF 5'UTR AND PROMOTER POLYMORPHISMS IN $P16^{cdkn2a}$ IN FOUR STRAINS OF INBRED RATS

Bartsch, Lois M, and Shull, James D.

Department of Genetics, Cell Biology, and Anatomy. Eppley Cancer Institute. University of Nebraska Medical Center, Omaha NE.

We have shown that the ACI rat is highly susceptible to 17 β -estradiol (E2)-induced mammary carcinoma, while the genetically related COP and the unrelated BN rat are not. We have also mapped to rat chromosome 5, *Emcal*, a genetic determinant of E2-induced mammary cancer in crosses between the ACI and either the COP or BN rats. $P16^{cdkn2a}$, a cell cycle regulator of the G1-S transition, maps within *Emcal*. The expression of $p16^{cdkn2a}$ is commonly down regulated in breast cancer in humans. Previous data from our lab has shown that $p16^{cdkn2a}$ protein is down regulated in focal regions of atypical hyperplasia. To begin to study any possible changes in gene regulation of $p16^{cdkn2a}$ between the ACI, BN and COP rats, we sequenced the 5'UTR and promoter region in four inbred rat strains. In total we sequenced 1900 bp of sequence that encompasses the 5'UTR and promoter of $p16^{cdkn2a}$ in the ACI, BN, COP and DA rats. We identified 16 polymorphisms between the four strains. We hypothesize that one or more of these polymorphisms alter the regulation of this gene in different strains of rats through changes in the frequency or timing of methylation. We have compared these sequences to the known mouse and human sequences to identify regions of the 5'UTR and promoter regions for similarities. In addition, we have used one of these polymorphisms to correlate $p16^{cdkn2a}$ genotype with the incidence of E2-induced mammary carcinoma in a phenotypically defined (BN x ACI) F_2 population. We have also begun to investigate how these polymorphisms may explain the phenotype differences in the susceptibility of the different strains of rats to E2-induced breast cancer with regard to transcriptional initiation and methylation of the promoter. Together, these data, strongly suggest that $p16^{cdkn2a}$ plays an early and contributory role in E2-induced mammary carcinogenesis in the ACI rat. Supported by NIH grant R01-CA77876, training grant DAMD17-00-1-0361 from the DOD, DOD grant DAMD17-03-1-0466.

Potential Early Role of $p16^{cdkn2a}$ in Estrogen-Induced Mammary Carcinogenesis in the ACI Rat

Lois M Beckerbauer, Benjamin Xie, Linda K Buckles, James D. Shull, Eppley Cancer Institute, University of Nebraska Medical Center, Omaha, NE

We have shown that the female ACI rat is highly susceptible to mammary carcinoma after prolonged exposure to 17 β -estradiol (E2), while the genetically related Copenhagen (COP) rat and the unrelated Brown Norway (BN) rat are not. More recently, we have mapped to rat chromosome 5, *Emcal1*, a determinant of susceptibility to E2-induced mammary cancer in F2 progeny from crosses between the ACI and COP or BN strains, *Cdkn2a* maps within *Emcal1*. $p16^{cdkn2a}$, one of at least two distinct products of *Cdkn2a*, regulates the G1 to S transition, and expression of this protein is commonly lost in breast cancers. Data from our laboratory indicate that expression of $p16^{cdkn2a}$ is significantly diminished in both focal regions of atypical hyperplasia and mammary cancers induced in ACI rats by E2, compared to the adjacent epithelium. To define further the role of $p16^{cdkn2a}$ in E2-induced mammary carcinogenesis, we are sequencing this gene from the ACI, COP and BN rat strains. Although no nucleotide polymorphisms have been identified within the coding region, several have been identified within the 5' flanking region. We hypothesize that these putative promoter polymorphisms may impact transcriptional regulation of $p16^{cdkn2a}$ and/or the probability that this gene will be transcriptionally silenced due to promoter methylation. Preliminary data suggest that $p16^{cdkn2a}$ mRNA expression is altered during E2-induced mammary carcinogenesis and that the promoter becomes methylated. A large phenotypically defined F2 population from a cross between BN females and ACI males was genotyped at one of the $p16^{cdkn2a}$ polymorphisms. Whereas 100% of F2 rats homozygous for ACI alleles at $p16^{cdkn2a}$ were mammary cancer positive following 196 days of E2 treatment, only 30% of those rats homozygous for BN alleles were tumor positive at this time point. Together, these data strongly suggest that $p16^{cdkn2a}$ plays an early and contributory role in E2-induced mammary carcinogenesis in the ACI rat. Supported by NIH grant RO1-77876 and training grant DAMB17-00-1-0361 from the DOD.